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Serovars, auxotypes, and plasmid contents of PPNG strains from outbreak in Amsterdam

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SUMMARY The first outbreak of penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) in Amsterdam in 1981-2 was caused mainly by African strains carrying the 24 megadalton transfer plasmid (Afr^+) that were non-requiring (NR) and inhibited by phenylalanine ($phen^i$), but African strains without the transfer plasmid (Afr^-) that were NR$phen^i$ and Afr^+ NR strains were also found.

Serological classification, using two monoclonal antibody systems, showed that three main serovars (Ae/Av, Aedih/Arst, and Bacejk/Brpyust) could be distinguished in these PPNG strains, which indicated exchanges of plasmids in these serovars. The serovar Ae/Av predominated in the Afr^+ and Bacejk/Brpyust in the Afr^- strains.

Introduction

Van Embden *et al* found that, in almost all the β lactamase (penicillinase) producing *Neisseria gonorrhoeae* (PPNG) strains isolated in Amsterdam in 1976-9, resistance to penicillin was encoded for by a 4·5 megadalton plasmid.[1] Most of these isolates also carried a 24 megadalton transfer plasmid. Sporadic isolates, most of which were imported from west Africa, carried a smaller 3·3 megadalton resistance plasmid. The number of patients infected with PPNG strains started to increase in October 1980 and reached a peak in January 1981. Until October 1980 no strain harbouring the 3·3 megadalton plasmid had been found to carry the transfer plasmid (Afr^- strains). Of 54 PPNG strains isolated in October 1980 and February to March 1981 and analysed, however, 38 harboured the 3·3 megadalton plasmid in conjunction with the 24 megadalton plasmid (Afr^+ strains).[2] The penicillin resistance was transferable to *Escherichia coli*, which indicated that the 3·3 megadalton plasmid was transferable when it coexisted with the 24 megadalton plasmid.

As the number of infections with PPNG strains in Amsterdam had increased steadily,[3] the Public Health Laboratory introduced routine auxotyping of the isolates in March 1982,[4] and the National Institute of Public Health started measuring plasmid profiles in April 1981.[5]

From March 1981 to September 1982, 341 Afr^+ non-requiring (NR) and inhibited by phenylalanine ($phen^i$) strains, 26 Afr^+ NR strains, and 106 Afr^- NR $phen^i$ strains were isolated.[4] These 473 strains constituted 65% of the 729 PPNG strains isolated in Amsterdam during this period. Only nine Afr^+ and two Afr^- strains belonged to other auxotypes. We undertook serological classification of a representative sample of the 473 NR strains into serovars to analyse further the outbreak of PPNG strains.

Materials and methods

PPNG STRAINS

We included in the study 208 (61%) of the 341 Afr^+ NR $phen^i$ PPNG strains, 99 (93%) of the 106 Afr^- NR $phen^i$ strains, and all 26 Afr^+ NR strains isolated in Amsterdam in March 1981 to September 1982.

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TABLE *Serovars of 333 non-requiring (NR) penicillinase producing strains of Neisseria gonorrhoeae (PPNG) with African type 3·3 megadalton plasmid related to coexistance with 24 megadalton transfer plasmid (Afr$^+$) and inhibition by phenylalanine (phen^i)*

	No (%) of strains classified as:		
GS/Ph serocombinations	*Afr$^+$ NR phen^i*	*Afr$^+$ NR*	*Afr$^-$ NR phen^i*
Ae/Av	172 (83)	26 (100)	4 (4)
Aedih/Arst	11 (5)		15 (15)
Bacejk/Brpyust	21 (10)		76 (76)
Bacejk/Brpyut			2 (2)
Other	4 (2)		2 (2)
Total	208	26	99

*Ph supplied by Pharmacia Diagnostics, Uppsala, Sweden, GS supplied by Syva, Palo Alto, USA.

METHODS

Identification of β lactamase production, auxanographic typing, and plasmid characterisation have been described previously.[4 5] Serological classification into serovars was performed as described[6] using two different sets of monoclonal antibodies, the GS antibodies (Syva, Palo Alto, USA) and the Ph antibodies (Pharmacia Diagnostics, Uppsala, Sweden).

Results

All 26 Afr$^+$ NR strains and most (172/208, 83%) of the Afr$^+$ NR phen^i strains belonged to the WI GS/Ph serovar Ae/Av (table and figure) in contrast to only 4% (4/99) of the Afr$^-$ NR phen^i strains. The GS serovar Bacejk dominated in the Afr$^-$ strains (79%, 78/99), whereas only 10% (21) of the 208 Afr$^+$ NR phen^i strains belonged to this GS serovar. The GS serovar Bacejk could be resolved into two Ph/GS serovar combinations (table), of which Bacejk/Brpyust accounted for 76% (76/99) of the Afr$^-$ strains and 10% (21/208) of the Afr$^+$ NR phen^i strains. Aedih/Arst was represented by 5% (11/208) of the Afr$^+$ strains and 15% (15/99) of the Afr$^-$ strains.

Only 2% (4/208) of the Afr$^+$ NR phen^i strains and 2% (2/99) of the Afr$^-$ NR phen^i strains belonged to other serovars.

Discussion

Earlier studies showed that the outbreak of gonorrhoea caused by PPNG strains in Amsterdam in 1981 was caused mainly by Afr$^+$ NR phen^i strains.[4 5] PPNG strains of the same type but without the transfer plasmid were also isolated during the same time, though in smaller numbers.[4] Serological classification of all these PPNG strains showed that three main serovars could be distinguished, Ae/Av, Aedih/Arst, and Bacejk/Brpyust (table). In all, Ae/Av strains accounted for 202 (61%) of the 333 tested PPNG strains isolated during the study period 1981–2. The serovar Ae/Av dominated in Afr$^+$ strains, and Bacejk/Brpyust in Afr$^-$ strains. Ae/Av NR phen^i strains with the 3·3 megdalton plasmid seemed to have a great ability to survive and be transmitted. This ability seemed to be greater in strains that also carried the transfer plasmid.

Two other PPNG outbreaks caused by Ae/Av strains have been reported; one in Sweden caused by Ae/Av NR strains that infected 31 patients, including

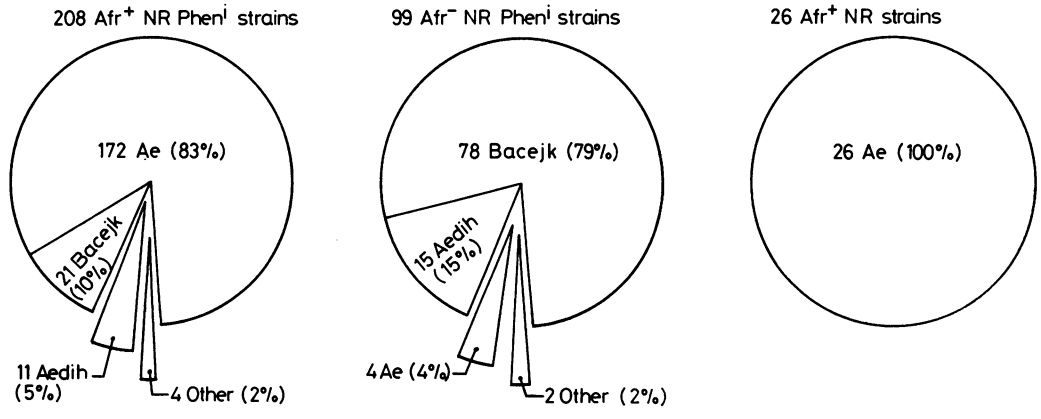

FIGURE *Serovars (classified with GS monoclonal antibodies (Syva, Palo Alto, USA)) of 333 non-requiring (NR) penicillinase producing strains of Neisseria gonorrhoeae (African strains with (Afr$^+$) or without (Afr$^-$) 24 megadalton transfer plasmid and inhibited by phenylalanine (phen^i)) isolated in Amsterdam 1981–2.*

six prostitutes,[7] and one in Canada caused by Asia$^+$ Ae strains requiring proline and ornithine (Pro$^-$ Orn$^-$).[8]

The Bacejk/Brpyust NR phen^i strains harbouring only the African type plasmid (Afr$^-$) may, however, be more easily transmitted than those (Afr$^+$) containing a coexisting 24 megadalton plasmid, or the 24 megadalton plasmid may have been introduced in strains of the NR phen^i phenotype late in the study period. Afr$^+$ strains had, however, been isolated by the start of the outbreak. The presence or absence of the 24 megadalton plasmid may confer a selective pressure that differentiates between strains of protein IA and protein IB serogroups.

Ae/Av NR phen^i strains with the Asian type plasmid were not identified in PPNG strains in Amsterdam during the study period,[4] and the serovar Ae/Av has not been identified in gonococcal strains from places like Bangkok or Singapore or in PPNG strains imported to Sweden from South East Asia (Bygdeman *et al*, unpublished data). The Afr$^+$ PPNG strains of the phenotype Ae/Av NR phen^i are therefore probably not the result of deletion of the 4·5 megadalton plasmid in Asia$^+$ strains but the acquisition of the 24 megadalton plasmid by Afr$^-$ strains. Aedih/Arst is the dominating WI serovar in South East Asia in both PPNG and non-PPNG strains, most of them being Pro$^-$. NR Aedih/Arst PPNG strains have, however, been imported to Sweden from South East Asia, but not from Africa (Bygdeman *et al*, unpublished data). The Afr$^+$ Aedih/Arst strains that caused the PPNG outbreak in Amsterdam may therefore have originated from Asia$^+$ PPNG strains.

The presence of the transfer plasmid increases the ability of the R plasmid to disseminate, as the transfer plasmid is capable of mobilising the non-autotransmissible R plasmid into other gonococci.[9] The number of different gonococcal strains, however, as judged by the number of serovars, was comparable in Afr$^+$ and Afr$^-$ strains.

Hendry and Dillon suggested that phenylalanine sensitive cells may promote replication or transmission of the 3·2 megadalton plasmid.[10] This might explain why only one serovar (Ae/Av) was seen in the Afr$^+$ NR strains, whereas five different serovars were found in the Afr$^+$ NR phen^i strains.

Plasmid profile assessment, auxanographic typing, and serological classification into serovars contribute to our understanding of the epidemiology, biological properties, and evolution of β lactamase producing gonococci.

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